

## The Presence of Only One of Five Exoribonucleases Is Sufficient To Support the Growth of *Escherichia coli*

KAREN OST KELLY† AND MURRAY P. DEUTSCHER\*

Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06030-3305

Received 24 July 1992/Accepted 12 August 1992

***Escherichia coli* contains multiple exoribonucleases. Strains lacking the exoribonucleases RNase II, D, BN, T, and PH are inviable. The introduction of a chromosomal, wild-type copy of the gene for any one of these enzymes is sufficient to allow cell growth, with the enzymes being in the following order of effectiveness: RNase T > RNase PH > RNase D > RNase II > RNase BN. The data indicate that these five exoribonucleases functionally overlap in vivo and that any one of them can take over the functions of all the others, although with various efficiencies.**

Seven exoribonucleases, distinct both genetically and biochemically, are now known to be present in *Escherichia coli* (3). Important questions that are raised by this unexpected multiplicity of RNases are whether each of the enzymes has a separate primary function in vivo, whether some serve as backup enzymes when others are absent, and how extensive the overlap in their specificities is. Earlier studies showed that mutations eliminating only a single exoribonuclease have little or no effect on growth (reviewed in references 2 and 3), indicating that for every one of these enzymes there is an alternate RNase that can effectively take over any of their required functions. Even certain multiple combinations of RNase mutations have only relatively minor growth defects (10, 11). Thus, a strain devoid of the exoribonucleases RNase II, D, BN, and T, as well as the nonspecific endoribonuclease RNase I, grows only slightly more slowly than the wild type (10). On the other hand, certain other combinations of mutations, such as in RNase II and polynucleotide phosphorylase (PNP) (6) or in RNase PH and PNP (7), can have major effects on growth.

We have recently shown that introduction of a null mutation in the *rph* gene, encoding RNase PH, into the aforementioned RNase I<sup>-</sup> II<sup>-</sup> D<sup>-</sup> BN<sup>-</sup> T<sup>-</sup> strain leads to an inviable phenotype (7). This finding has provided us with a system to determine the degree of functional overlap in vivo among the five exoribonucleases RNase II, D, BN, T, and PH. To accomplish this, we have constructed strains in which only one of the five enzymes is still present and have examined the growth properties of the individual strains. Our results indicate that any one of the five exoribonucleases is sufficient to maintain viability of *E. coli* cells but that their growth properties vary widely. These results demonstrate a surprisingly high degree of functional overlap among these enzymes in vivo.

*E. coli* cells containing only one of the five exoribonucleases under study were constructed by phage P1-mediated transduction. Strain CAN20-12E *rnt::kan*, lacking RNases I, II, D, BN, and T, and strain CAN20-12E/18-11, lacking RNases I, II, D, and BN and containing 30% of RNase T (T<sup>+/−</sup>), were previously described (5, 9); the former strain served as the RNase PH<sup>+</sup> cell. This strain was transduced to

either RNase II<sup>+</sup>, RNase D<sup>+</sup>, or RNase T<sup>+</sup> by using nearby *Tn10* insertions and assaying the Tc<sup>r</sup> transductants for the RNase activity of interest. The resulting strains and CAN20-12E/18-11 were then converted to RNase PH<sup>−</sup> by transduction with *rph::cam*, obtained from strain K4716 (1), provided by David Friedman, University of Michigan. This procedure served for the construction of the RNase II<sup>+</sup>, RNase D<sup>+</sup>, RNase T<sup>+</sup>, and RNase T<sup>+/−</sup> strains. The strain containing RNase BN as the only one of the five exoribonucleases was made from strain CA265/3-1 (RNase II<sup>−</sup> D<sup>−</sup>) (4) by introduction of *rnt::kan* and *rph::cam*. The *rna* mutation was introduced by using a nearby *Tn10* after first converting the strain to Tc<sup>s</sup> and assaying Tc<sup>r</sup> transductants for RNase I activity. The mutations in *rnd*, *rnt*, and *rph* are null mutations, whereas those in *mb* and *rbn* (RNase BN) have not been characterized structurally but display very low levels of activity of the relevant enzyme (1% or less, on the basis of enzyme assay or ability to plate phage).

The identities of the multiple-RNase-deficient strains were confirmed by assay of extracts for each of the five RNase activities under study. The substrates used for the assays were as follows: RNase II, [<sup>3</sup>H]poly(A); RNase D and RNase PH, tRNA-CCA-[<sup>3</sup>H]C<sub>2-3</sub>; RNase T, tRNA-CC[<sup>14</sup>C]A; and RNase BN, tRNA-C[<sup>14</sup>C]A. For the RNase PH assay, P<sub>i</sub>-dependent activity was determined. Conditions for the various assays were as described previously (4, 9) and in Table 1, footnote a.

The data in Table 1 show that for each of the multiple-RNase-deficient strains the RNase activities are as expected. Thus, for four of the strains (T<sup>+</sup>, PH<sup>+</sup>, D<sup>+</sup>, and II<sup>+</sup>), the one RNase in each expected to be at a high level does display a level of activity close to that of the wild type on the appropriate substrate, whereas levels of activity against all the other substrates are much lower. However, it is also evident that the mutant extracts can have considerable activity against other tRNA substrates, indicating a considerable amount of overlap in substrate specificity, as is already known (10). In contrast, RNase II is the only RNase that acts on poly(A) to a significant degree, and RNase PH is the only enzyme of the five that displays a significant level of P<sub>i</sub>-dependent activity.

The identity of the BN<sup>+</sup> strain could not be confirmed by assay alone because all the RNases act on the substrates for RNase BN, tRNA-CA or tRNA-CU, to some degree, and RNase BN has a relatively low level of activity (11). Never-

\* Corresponding author.

† Present address: Department of Biochemistry, Stanford University, Stanford, CA 94305.

TABLE 1. Exoribonuclease assays of multiple-RNase-deficient strains<sup>a</sup>

Relevant phenotype <sup>b</sup>	Relative sp act (%) with the following substrate (RNase assay)				
	tRNA-CC[ <sup>14</sup> C]A (T)	tRNA-CCA-[ <sup>3</sup> H]C <sub>2-3</sub> + P <sub>i</sub> (PH)	tRNA-CCA-[ <sup>3</sup> H]C <sub>2-3</sub> (D)	[ <sup>3</sup> H]Poly(A) (II)	tRNA-C[ <sup>14</sup> C]A (BN)
Wild type	100	100	100	100	100
I <sup>-</sup> II <sup>-</sup> D <sup>-</sup> BN <sup>-</sup> PH <sup>-</sup> (T <sup>+</sup> )	100	<6	24	6	8
I <sup>-</sup> II <sup>-</sup> D <sup>-</sup> T <sup>-</sup> BN <sup>-</sup> (PH <sup>+</sup> )	14	106	22	1	11
I <sup>-</sup> II <sup>-</sup> T <sup>-</sup> BN <sup>-</sup> PH <sup>-</sup> (D <sup>+</sup> )	34	<6	115	1	8
I <sup>-</sup> D <sup>-</sup> T <sup>-</sup> BN <sup>-</sup> PH <sup>-</sup> (II <sup>+</sup> )	32	14	42	102	20
I <sup>-</sup> II <sup>-</sup> D <sup>-</sup> T <sup>-</sup> PH <sup>-</sup> (BN <sup>+</sup> )	12	8	15	4	10

<sup>a</sup> Sonicated extracts were made as previously described (8). RNases were assayed under standard conditions as previously described (4, 8). Reaction mixtures for RNases T, PH, D, and BN were all incubated at 37°C for 30 min with various amounts of extract and substrate, as follows: RNase T, 20 µl of extract and 12 µg of tRNA-CC[<sup>14</sup>C]A; RNase PH, 10 or 20 µl of extract and 10 µg of tRNA-CCA-[<sup>3</sup>H]C<sub>2-3</sub>; RNase D, 40 µl of extract and 5 µg of tRNA-CCA-[<sup>3</sup>H]C<sub>2-3</sub>; RNase BN, 50 µl of extract and 10 µg of tRNA-C[<sup>14</sup>C]A. RNase II was assayed against [<sup>3</sup>H]poly(A) with 10 µl of sonicated extracts in samples which were incubated at 37°C for 10 min.

<sup>b</sup> Symbols in parentheses indicate which RNase is present.

theless, the activity assays showed that the BN<sup>+</sup> strain is lacking RNases T, PH, D, and II (Table 1). That the BN<sup>+</sup> strain actually contained RNase BN was shown by its ability to plate the amber-*psu*<sub>1</sub><sup>+</sup> T4 phage BU33. This phage cannot form plaques on an RNase BN<sup>-</sup> strain (8). Thus, each of the strains contains only one of the five exoribonucleases.

From the foregoing discussion of strain construction and assay of RNase activities, it is evident that while cells lacking the five exoribonucleases do not survive (7), cells containing only one of the five are viable. In fact, a strain lacking RNases I, II, D, BN, and PH and 70% of RNase T is viable as well (Table 2). Thus, each one of the five exoribonucleases has sufficient breadth of specificity *in vivo* to take over the functions and substitute for the other four missing enzymes.

To determine the relative effectiveness with which each of the RNases can substitute for all five of the enzymes, we determined the growth rate of each of the strains containing only a single exoribonuclease. As shown in Table 2, although any one of the RNases can support growth, they do so with very different efficiencies. On the basis of this criterion, RNase T has the broadest specificity, maintaining a cell doubling time of 35 min compared with 24 min for the wild-type strain containing all of the RNases. Even a cell with only 30% of the normal level of RNase T maintains relatively effective growth, doubling every 57 min. It should be noted that RNase T is the only one of the five exoribo-

nucleases whose removal by a single mutation does affect growth (10). Thus, although RNase T can substitute reasonably well when all four of the other RNases are missing, the other four enzymes, even in combination, cannot completely take over the role of RNase T.

For the other four exoribonucleases, their order of effectiveness in supporting growth is as follows: RNase PH > RNase D > RNase II > RNase BN. For the most slowly growing strain, the BN<sup>+</sup> strain, measurement of accurate doubling times was difficult because of the tendency of this cell to revert to a faster-growing form. The value reported is for the lowest growth rate observed in several growth experiments. The nature of the change(s) resulting in faster growth is presently unknown, but analysis of the possible suppressor mutation(s) will be of considerable interest.

The data presented here demonstrate that any one of the five exoribonucleases is sufficient to overcome the no-growth phenotype of an RNase I<sup>-</sup> II<sup>-</sup> D<sup>-</sup> BN<sup>-</sup> T<sup>-</sup> PH<sup>-</sup> cell, indicating a high degree of functional overlap among these enzymes *in vivo*. However, the enzymes vary considerably in their ability to support growth, as would be expected from the different substrate specificities of these enzymes *in vitro* (3). Nevertheless, this situation is quite different from that observed with genes encoding some of the *E. coli* endoribonucleases in which single mutations can have profound effects on cell growth (2). Why *E. coli* requires the presence of so many exoribonucleases is unclear. Presumably, there are certain situations encountered in nature, which are not reproduced by the growth in rich media in the laboratory, for which the presence of all of these enzymes provides a selective advantage.

Finally, it should be noted that two other exoribonucleases, PNP and RNase R, are known to be present in *E. coli* (2, 3). Clearly, these two enzymes by themselves are not sufficient to support the growth of a cell. However, they may contribute to the ability of the strains containing one of the five remaining exoribonucleases to grow, especially if the substrate specificities of PNP and RNase R complement that of the other RNase. Examination of the properties of strains containing additional combinations of mutations, including those in genes encoding PNP and RNase R, should help to sort out which RNases are the most effective backups for others and what the primary functions of each of the enzymes *in vivo* are.

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TABLE 2. Growth rates of strains containing one exoribonuclease

Relevant phenotype <sup>a</sup>	Doubling time (min) <sup>b</sup>
Wild type .....	24
I <sup>-</sup> II <sup>-</sup> D <sup>-</sup> T <sup>-</sup> BN <sup>-</sup> PH <sup>-</sup> .....	Invisible <sup>c</sup>
I <sup>-</sup> II <sup>-</sup> D <sup>-</sup> BN <sup>-</sup> PH <sup>-</sup> (T <sup>+</sup> ) .....	35
I <sup>-</sup> II <sup>-</sup> D <sup>-</sup> T <sup>-</sup> BN <sup>-</sup> (PH <sup>+</sup> ) .....	40
I <sup>-</sup> II <sup>-</sup> T <sup>-</sup> BN <sup>-</sup> PH <sup>-</sup> (D <sup>+</sup> ) .....	64
I <sup>-</sup> D <sup>-</sup> T <sup>-</sup> BN <sup>-</sup> PH <sup>-</sup> (II <sup>+</sup> ) .....	70
I <sup>-</sup> II <sup>-</sup> D <sup>-</sup> T <sup>-</sup> PH <sup>-</sup> (BN <sup>+</sup> ) .....	105
I <sup>-</sup> II <sup>-</sup> D <sup>-</sup> BN <sup>-</sup> PH <sup>-</sup> (T <sup>+</sup> /-) .....	57

<sup>a</sup> Symbols in parentheses indicate which RNase is present.

<sup>b</sup> Overnight cultures were diluted 1:200 into 20 ml of yeast extract-tryptone medium supplemented with 0.2% glucose and were grown at 37°C. The A<sub>550</sub> was measured at 30-min intervals.

<sup>c</sup> Taken from reference 7.

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